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Genome-wide meta-analysis identifies genetic variants associated with glycemic response to sulfonylureas.

Running title: GWAS of glycaemic response to sulfonylureas.

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Abstract

Objective: Sulfonylureas, the first available drugs for the management of type 2 diabetes, remain widely prescribed today. However there exists significant variability in glycaemic response to treatment. We aimed to establish heritability of sulfonylurea response and identify genetic variants and interacting treatments associated with HbA1c reduction.

Research design and methods: As an initiative of the Metformin Genetics Plus (MetGen Plus) and the DIabetes REsearCh on patient stratiFication (DIRECT) consortia, 5,485 white Europeans with type 2 diabetes treated with sulfonylurea were recruited from 6 referral centres in Europe and North America. We first estimated heritability using generalized restricted maximum likelihood (REML) and then undertook GWAS of glycemic response to sulfonylureas measured as HbA1c reduction after 12 months of therapy followed by meta-analysis. These results were supported by acute glipizide challenge in humans who were naïve to type 2 diabetes medications, cis-eQTLs and functional validation in cellular models. Finally, we examined for a possible drug-drug-gene interactions.

Results: After establishing that sulfonylurea response is heritable ($37 \pm 11\%$), we identified two independent loci near the *GXYLT1* and *SLCO1B1* genes associated with HbA1c reduction at a genome-wide scale ($p < 5 \times 10^{-8}$). The C-allele at rs1234032, near *GXYLT1*, was associated with 0.14% (1.5 mmol/mol), $p = 2.39 \times 10^{-8}$ lower reduction in HbA1c. Similarly, the C-allele was associated with higher glucose trough levels ($\beta = 1.61$, $p = 0.005$) in healthy volunteers in the SUGAR-MGH given glipizide ($N = 857$). In 3,029 human whole blood samples, the C-allele is a cis-eQTL for increased expression of *GXYLT1* ($\beta = 0.21$, $p = 2.04 \times 10^{-58}$). The C-allele of rs10770791, in an intronic region of *SLCO1B1*, was associated with 0.11% (1.2 mmol/mol) greater reduction in HbA1c ($p = 4.80 \times 10^{-8}$). In 1,183 human liver samples, the C-allele at rs10770791 is a cis-eQTL for reduced *SLCO1B1* expression

($p=1.61\times 10^{-7}$) which, together with functional studies in cells expressing *SLCO1B1*, supports a key role for hepatic *SLCO1B1* (encoding OATP1B1) in regulation of sulfonylurea transport. Further, a significant interaction between statin use, sulfonylurea response and *SLCO1B1* genotype was observed ($p=0.001$). In statin non-users, C-allele homozygotes at rs10770791 had a large absolute reduction in HbA1c ($0.48\pm 0.12\%$ (5.2 ± 1.26 mmol/mol)), equivalent to initiating a DPP4 inhibitor.

Conclusion: We have identified clinically important genetic effects at genome wide levels of significance, and important drug-drug-gene interactions, which include commonly prescribed statins. With increasing availability of genetic data embedded in clinical records these findings will be important when prescribing glucose-lowering drugs.

Introduction

Sulfonylureas are potent glucose-lowering drugs that reduce HbA1c by an average of 1.5% (18 mmol/mol) (1). Despite an increasing trend to use more modern, expensive treatments, sulfonylureas remain commonly prescribed in the UK, making up 27% of new prescriptions, second only to metformin (2). Due to their very low cost, they are extensively used in low- and middle-income countries. However, considerable variation exists in response to sulfonylureas, with 10-20% of people with diabetes not responding at initiation of sulfonylurea therapy and 30-35% failing to respond to monotherapy after 5 years (3, 4). It is likely that a combination of genetic and non-genetic modifying factors underlies the clinical variability of glycaemic response to sulfonylureas. While many clinical risk factors such as baseline HbA1c, sex, duration of diabetes and dose are associated with glycaemic response to sulfonylureas (5-7), modulatory genetic factors remain largely unexplored with the exception of a few proof-of-concept studies using a candidate gene approach (8-12).

Glycaemic response to metformin is heritable with 34% of the variance in response explainable by common genetic variants (13-15). There have been no similar estimates for sulfonylurea response and to date, no genome-wide association studies (GWAS) of glycaemic response to sulfonylurea treatment have been reported, so the genetic contribution to how patients respond to sulfonylureas and clinical implication of this genetic variation has not been systematically studied. As an initiative of the Metformin Genetics Plus (MetGen Plus) and the DIabetes REsearch on patient strATification (DIRECT) consortia, we report here the first genome-wide meta-analysis of glycaemic response to sulfonylureas measured as HbA1c reduction after 12 months of therapy. Based upon these findings we then explore the impact of interacting drugs and identify clinically important genotype dependent statin-sulfonylurea interactions for this important class of diabetes therapies.

Methods

List of abbreviations used throughout this article and their corresponding explanations are shown in Supplementary Table 1.

Study design and participants

We established an international consortium allowing recruitment of 5,485 unrelated individuals of European ancestry from six referral centers in Europe and North America as part of the MetGen and DIRECT consortia (Supplementary Table 2). Included participants had a clinical diagnosis of type 2 diabetes and were treated with sulfonylureas as monotherapy or as an add-on to metformin. This study was approved by respective research ethics review boards and participants provided written informed consent.

Sample ascertainment

Clinical, prescription and biochemical data were retrieved from the electronic medical record systems. Participants with type 2 diabetes aged more than 35 years at diagnosis who used sulfonylureas with no history of insulin use were ascertained. They were stably treated with sulfonylureas for at least six months with no other glucose-lowering drug started or stopped within the study period. The baseline HbA1c was between 7% (53.0 mmol/mol) and 14% (129.5 mmol/mol) at sulfonylurea initiation.

Measurement of glycemic response and definition of variables

Participants' glycemic response to sulfonylurea was modelled as the quantitative phenotype of HbA1c reduction between baseline HbA1c and treatment HbA1c while the patients were maintained on stable treatment. Baseline HbA1c was defined as the closest HbA1c measure

to sulfonylurea initiation and within six months before and seven days after this date. The treatment HbA1c was the closest HbA1c measure to 12 months after initiation of sulfonylureas (between 6 and 15 months).

In all the studies, covariates were selected based on previous reports and univariate association between the outcome variable (HbA1c reduction) and explanatory variables. The best fit linear regression model was determined using stepwise backward elimination.

Accordingly, baseline HbA1c, sex, age at diagnosis, baseline BMI, average daily dose, time between baseline HbA1c and treatment HbA1c and drug group (sulfonylurea monotherapy or sulfonylurea added to metformin) were considered in the final model as available in each cohort (Supplementary Table 3). Average daily dose was calculated as the mean daily dose of prescriptions filled during the study period (mean of percentage of each sulfonylurea divided by maximum prescribable according to the British National Formulary). Baseline weight was the nearest measure to the sulfonylurea start date (index date) and within 180 days on either side of the index date. Each study was adjusted for the top n principal components (PCs) to account for 80~90% of the variation in population structure.

The final response model was: $\text{HbA1c reduction} \sim \text{baseline HbA1c} + \text{PCs} + \text{study specific covariates}$.

Genome-wide array genotyping, quality control and imputation

Each respective cohort performed genome-wide genotyping on a variety of arrays as illustrated in Supplementary Table 3. Genotyping and quality-control procedures for the GoDARTS, DCS, and PMET cohorts has been previously described (13, 15, 16). Genotyping data for each platform were individually cleaned by each study center. Standard post-genotyping quality-control procedures were applied to each data set (Supplementary Figure

1). Monomorphic, SNPs with minor allele frequency (MAF) <1% or call rate <98% or Hardy-Weinberg equilibrium (HWE) <10⁻⁶ were removed. Samples with genotyping calls <98% or heterozygosity >3 standard deviation from the mean or correlated with another sample (Identity by descent >0.125) were filtered out. All genetic variants were mapped to and reported using Genome Reference Consortium Human genome build 37 (GRCh37). Each data set was then imputed to the 1000 Genome CEU reference panel (phase 1, version 3) with IMPUTE software (17), except PMET2 and Geisinger where imputation was performed using the HRC.r1-1 EUR reference genome (GRCh37 build) using the Michigan server. Post-imputation, SNPs with poor imputation quality (Info < 0.6), monomorphic variants or MAF < 5% were excluded (Supplementary Figure 1).

Genome-wide association analysis

Following imputation, each respective cohort conducted GWAS under an additive genetic model to assess the role of common variants (MAF ≥ 5%) in glycemic response to sulfonylureas. Each SNP was tested for association with quantitative measure of sulfonylurea related HbA1c reduction with SNPTEST v2.536 (18) using multiple linear regression correcting for baseline HbA1c, genotypic PCs and other study specific variables (Supplementary Table 3). Genome-wide association analyses were carried out separately by respective study centers. Prior to meta-analysis, we performed post-GWAS harmonization and QC of GWAS results from each cohort to track possible errors in the study-specific analyses. We used the standard protocol accompanied by EasyQC R package (19). Specifically, we removed SNPs with MAF < 5%, low imputation quality (< 0.6), large absolute values of beta coefficients and standard errors (≥10), low call rate (< 0.98), and deviations from HWE ($p < 10^{-6}$). Meta-analysis was then performed using an inverse

variance weighted fixed effect model, implemented in GWAMA v2.1.34 (20). Post meta-analysis, SNPs with MAF < 5%, available in less than six studies, with large absolute values of beta coefficients and standard errors (≥ 10) were excluded (Supplementary Figure 1). Heterogeneity was assessed using the I^2 metric from the complete study-level meta-analysis. Between-study heterogeneity was tested using the Cochran Q statistic and considered significant at $p < 0.1$. We used the commonly accepted threshold of 5.0×10^{-8} for joint p values to determine statistical significance. Nominal significance was considered to be $p < 0.05$. The CMplot package (21) in R was used to generate Manhattan and quantile-quantile plots. Regional plots around genome-wide or suggestive genes were visualized using LocusZoom (22). The final meta-analysis included 5,385,635 common autosomal SNPs from 5,485 independent individuals of European ancestors treated with sulfonylureas ($\lambda=1.008$) (Supplementary Figure 2).

Common variant heritability

We used the generalized restricted maximum likelihood (REML) approach under the LDAK assumptions using SumHer v5.1 (23) to estimate how much of the variance in HbA1c reduction after sulfonylurea treatment could be attributed to common genetic variants (SNP-based heritability, h^2 SNP). This method is a valid approach for estimating heritability in studies in which familial data with the same diagnosis who have received the same medication and assessed using the same treatment outcome is not feasible. In addition, SumHer uses GWAS summary without requiring individual-level data (23). Therefore, we estimated the SNP-heritability using summary statistics from the meta-GWAS. In order to avoid the impact of extreme linkage disequilibrium (LD) regions and disproportionately large effect size SNPs on heritability estimates, we exclude SNPs within the MHC (Chromosome

6: 25-34 Mb) and SNPs which individually explain >1% of phenotypic variation and SNPs in LD with these (within 1 cM).

Conditional Analysis

Given rs10770791 is in partial LD with previously established nonsynonymous variants, rs4149056 (*5; V174A, $D'=1$; $r^2=0.17$) and rs2306283 (*1B; N130D, $D'=0.98$; $r^2=0.63$), we performed conditional analysis by including these SNPs in the model together. This analysis was carried out with individual-level data from the GoDARTS and PMET cohorts (65% of the total population) and baseline HbA1c, PCs and other study specific covariates.

Biochemical response to glipizide

To test whether meta-GWAS identified genetic variants are associated with trough glucose levels, we performed a lookup using data from the Study to Understand the Genetics of the Acute Response to Metformin and Glipizide in Humans (SUGAR-MGH). SUGAR-MGH enrolled 1,000 participants at risk of anti-diabetic therapy in the future or individuals with lifestyle-controlled type 2 diabetes who are naïve to treatment. Participants received a single dose of 5 mg glipizide followed by measurement of glucose and insulin levels at 30, 60, 90, 120, 180, and 240 minutes. This was used to construct phenotypes of acute glipizide response. The association between rs1234032 and rs10770791 with glipizide response was performed using linear regression with baseline glucose, age, sex, and the first 10 PCs as a covariate (see supplementary note).

Drug-drug gene interaction analysis

Given we have identified a genetic variant in the *SLCO1B1* (a gene encoding hepatic transporter of statins) associated with glycaemic response to sulfonylureas, we checked for interaction between *SLCO1B1*-rs10770791 and statin use in a drug-drug-gene interaction model using linear regression, with HbA1c reduction as the dependent variable. This analysis was performed using individual-level data from the GoDARTS and PMET cohorts where we have access to prescription data.

Statin treated cases were recipients of sulfonylurea who were also prescribed statins for at least the three months prior to the measurement of treatment HbA1c. Statin untreated controls were those recipients of sulfonylurea who did not receive a statin prescription at least for one year prior to measurement of the treatment HbA1c.

Expression quantitative trait locus lookups

Expression quantitative trait loci (eQTL) analysis seeks to identify genetic variants that affect the expression of one or more genes: a gene-SNP pair for which the expression of the gene is associated with the allelic configuration of the SNP is referred to as an eQTL. eQTL lookups were performed in human liver and whole blood samples for rs10770791 and rs1234032, respectively. Additional lookups were performed using publicly available data from the GTEx consortium.

The human liver eQTL lookups were carried out using data from previous study performed by Dr. Federico Innocenti's group (24). In brief, this eQTL study was performed with 1,183 liver samples, combined from four dataset (24). We looked up the top associated SNP, rs10770791, from this study, as it is in the *SLCO1B1* and *SLCO1B3* region, which are genes that are abundantly expressed in the liver.

The human whole blood eQTL lookup was performed using data from the DIRECT consortium in a total of 3, 029 subjects who are at high risk of developing type 2 diabetes or recently diagnosed type 2 diabetes (25). Detailed explanation of the eQTL analysis is previously published (26) and summary statistics are available in the following DOI: 10.5281/zenodo.4475681

Cell culture and *in vitro* transport and inhibition studies

HEK293 Flp-In cells stably expressing empty vector (EV), OATP1B1, OATP1B3, were used to perform *in vitro* transport and inhibition studies to establish the potency of inhibitors as IC₅₀ (i.e., concentration of inhibitor required to inhibit 50% uptake of a particular OATP1B1 and OATP1B3 substrate). Stably transfected HEK-293 Flp-In cells were maintained in Dulbecco's Modified eagle medium H-21 medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 units/mL streptomycin, and 500 µg/mL Geneticin. For transport studies, 150,000 cells/well were seeded the day before experiment on a poly-d-lysine coated 48-well plate. After 16-24 hours, media were removed and cells were incubated at 37°C for 5-10 min in 0.5 mL Hank's Balanced Salt Solution (HBSS) (ThermoFisher Scientific). Uptake studies were initiated after removing 0.3 mL HBSS above and adding 0.15 mL of HBSS containing trace amount of ³H-glyburide (Perkin Elmer, NET1024250UC)) or ³H-glipizide (Moravek, MT1855) or ³H-esterone sulfate (as positive control, Perkin Elmer NET203250UC). After 5 minutes, radioactive substrates were removed and washed twice with 1 mL of ice cold HBSS. For inhibition studies, same methods above were used, where ³H-glyburide was used as substrate and various concentrations of atorvastatin (Cayman) or simvastatin (Cayman) were added together with ³H-glyburide. To compare the uptake of ³H-glyburide and ³H-glipizide in OATP1B1

reference and OATP1B1-174A (*5) expressing cells, studies were performed using stable and transiently transfected cells. The stable and transient experiments were carried out using HEK293 Flp-In cell lines expressing EV, OATP1B1-reference and OATP1B1-174A (*5) previously established by our group (27). These cell lines were used to determine the uptake of ^3H -glyburide, ^3H -glipizide and 3H-esterone sulfate (as positive control). In brief, each well was transfected with 200 ng of the DNA vector with 0.4 μL of Lipofectamine LTX transfection reagent (ThermoFisher) in a 48-well poly-d-lysine coated plate. Uptake studies were then performed after 48 hours using the methods described above and in triplicate wells.

Results

Glycemic response to sulfonylureas is heritable

The SNP heritability estimate (h^2) for a model-adjusted absolute reduction in HbA1c was $37\pm 11\%$, comparable to our previous estimate for metformin ($h^2=34\%$) (14). This suggests that about one third of the total variance of glycemic response to sulfonylureas is due to the additive effects of common variants.

GWAS identifies two variants associated with altered glycemic response to sulfonylureas

Meta-GWAS identified two genome-wide significant variants, rs1234032 and rs10770791, both on chromosome 12 (Figure 1, Supplementary Figure 2, Table 1). The most significant association was obtained for rs1234032, with a $-0.14\pm 0.03\%$ (-1.5 ± 0.3 mmol/mol) difference in HbA1c reduction per C-allele, $p=2.39\times 10^{-8}$. No statistical evidence for difference in effect size between studies was observed ($p_{het} = 0.55$) (Figure 3). We then examined data from a healthy volunteer population (SUGAR-MGH, $N = 857$) given a single dose of glipizide (28)

and found that the C-allele of rs1234032 was associated with higher post-dose glucose trough levels ($\beta=1.61$, $p=0.005$), and thus worse response, consistent with our GWAS findings. rs1234032 is an intergenic SNP, near *GXYLT1* (Figure 2, Figure 3), a gene that encodes a xylose transferase. rs1234032 is a cis-eQTL to *GXYLT1* in the whole blood using 3, 029 samples from the DIRECT consortium, with the C-allele being associated with increased expression ($\beta=0.21$, $p=2.04\times10^{-58}$). rs1234032 also showed a significant association with *GXYLT1* expression in multiple tissues including adipose subcutaneous ($p=8.1\times10^{-5}$), artery tibial ($p=2.8\times10^{-9}$), artery aorta ($p=3.4\times10^{-6}$), nerve tibial ($p=3.6\times10^{-6}$) and whole blood ($p=0.01$) from the GTEx consortium (29), with the C-allele associated with increased expression. These significant eQTL analyses could be due to strong linkage of rs1234032 ($D'=1$ and $R^2=0.95$) to rs7958582, which is within the cis-regulatory elements (<https://screen.wenglab.org/>). The C-allele of rs1234032 is also in LD with the A-allele of rs7964383 ($D'=0.98$, $r^2=0.41$), which is highly associated with increased whole blood gene expression ($p=1.7\times10^{-4}$) (29) and circulating protein levels of GXYLT1 (30). Both rs7958582 (β per G allele = -0.10, $p = 1.84\times10^{-06}$) and rs7964383 (β per A allele = -0.06, $p = 0.003$) were also nominally associated with glycaemic response to sulfonylureas.

The second variant, rs10770791, is located in an intron of *SLCO1B1* (Figure 2), and each copy of the C-allele (frequency 49.8%) was associated with a $0.11\pm0.02\%$ (1.2 ± 0.2 mmol/mol) greater HbA1c reduction, $p=4.80\times10^{-8}$. Stratified analyses showed a consistent direction of association across cohorts with similar effect sizes with no significant heterogeneity ($p_{\text{het}}=0.94$) (Figure 3). rs10770791 genotype was not significantly associated with sulfonylurea dose modification ($p=0.16$) or drug group (the likelihood of being on mono or dual therapy) ($p=0.29$). No significant association between rs10770791 and post-glipizide

trough glucose concentration was observed in healthy volunteers given glipizide in SUGAR-MGH ($\beta = -0.37$, $p = 0.46$).

rs10770791 is an eQTL for SLCO1B1 that encodes OATP1B1, a transporter of sulfonylureas

Focusing on the *SLCO1B1* locus, we performed locus-wide meta-analysis to identify the candidate causal gene (Figure 2). We also examined two established common nonsynonymous variants in *SLCO1B1*, rs4149056 (*5; V174A) and rs2306283 (*1B; N130D) (30). rs4149056 ($D'=1$; $r^2=0.17$) and rs2306283 ($D'=0.98$; $r^2=0.63$) were in partial LD with rs10770791, with both rs4149056 ($\beta=0.10\pm0.03\%$ (1.1 ± 0.3 mmol/mol), $p=2.72\times10^{-4}$) and rs2306283 ($\beta=0.08\pm0.02\%$ (0.9 ± 0.2 mmol/mol), $p=4.32\times10^{-5}$) nominally associated with sulfonylurea response. However, in a conditional analysis where we have individual level data from the GoDARTS and PMET cohorts, $n=3,557$ (65% of the total population), only rs10770791 remained strongly associated with sulfonylurea response ($\beta=0.15\pm0.05\%$ (2 ± 0.4 mmol/mol), $p=1.4\times10^{-3}$); with rs4149056 ($\beta=0.03\pm0.05\%$ (0.3 ± 0.4 mmol/mol), $p=0.58$) and rs2306283 ($\beta=0.06\pm0.05\%$ (0.7 ± 0.4 mmol/mol), $p=0.19$) not significant.

We then undertook eQTL lookups of *SLCO1B1* expression in 1,183 liver samples of European ancestry (24) and demonstrated that the C-allele of rs10770791 was associated with decreased *SLCO1B1* expression ($\beta = -5.24$, $p=1.61\times10^{-7}$) and marginally with decreased *SLCO1B3* expression ($\beta = -2.46$, $p=0.01$). We found directionally consistent but non-significant associations in the 208 liver samples examined by the GTEx project ($\beta = -0.06$, $p = 0.13$ for *SLCO1B1*).

Glyburide is a substrate of both OATP1B1 and OATP1B3 (31-35), whereas there are conflicting reports about glipizide, which has been shown to be a substrate of OATP1B3, but not OATP1B1 (31). We therefore undertook functional studies on sulfonylurea transport and observed that both glyburide and glipizide were substrates of OATP1B1 and OATP1B3 in HEK293 cells recombinantly expressing the transporters (Figure 4A). Further, we observed that OATP1B1-Ala174 (c.521C) had a significantly lower uptake of glyburide ($p<0.002$) and a trend towards a lower uptake of glipizide ($p=0.06$) compared to OATP1B1-Val174 (c.521T) (Figure 4B).

Statins inhibit sulfonylurea transport via OATP1B1; genetically reduced OATP1B1 transport has a large effect in non-statin users.

Given the high frequency with which hypercholesterolaemia and diabetes co-occur, statins are often taken concomitantly with sulfonylureas. OATP1B1, expressed on the basolateral membrane of human hepatocytes (36), contributes to the hepatic uptake of sulfonylureas and statins from portal blood (37). We therefore sought to examine whether the initiation of statins in patients receiving sulfonylurea is associated with glycemic response in a drug-drug-gene interaction model with a sample of 3,566 adults, where we have access to individual level data. Using retrospective data from the GoDARTS and PMET cohorts; 2,096 (59%) sulfonylurea users were co-prescribed statins and 1,470 (41%) were not. In a multiple linear regression model adjusted for baseline HbA1c, statin co-treatment was associated with greater HbA1c reduction on initiation of sulfonylurea, but only when adjusted for rs10770791 ($0.22\pm0.09\%$ (2 ± 1.0 mmol/mol), $p=0.02$). These results highlight a significant interaction between statin use and *SLCO1B1* genotype (rs10770791) ($p=0.001$) (Supplementary Table 4). In support of these results, we show that atorvastatin acid and simvastatin acid inhibited

OATP1B1 and OATP1B3-mediated uptake of glyburide with IC_{50} values ranging between 0.2 and 2.9 μ M (Supplementary Table 5), consistent with previous studies showing that these two statins inhibit OATP1B1-mediated uptake of estradiol-17 β -glucuronide (38).

We then performed stratified analysis to see if statin use modifies the association between rs10770791 and sulfonylurea related HbA1c reduction using a similar model. We observed that the effect of rs10770791 was abolished in sulfonylurea users prescribed statins ($\beta=0.053\pm0.03\%$ (0.6 ± 0.3 mmol/mol), $p=0.11$). However, among users of sulfonylureas without statins, we found a pronounced HbA1c reduction associated with the C-allele of rs10770791 ($\beta=0.23\pm0.049\%$ (2.4 ± 0.6 mmol/mol), $p=3.1\times10^{-6}$) (Supplementary Table 6). C-allele homozygotes at rs10770791 had a $0.48\pm0.12\%$ (5.2 ± 1.26 mmol/mol) greater absolute HbA1c reduction than T-allele homozygotes.

Discussion

We report the first meta-GWAS on glycemic response to sulfonylureas and establish that this trait is heritable with a 37% heritability estimate. We have identified two novel loci at chromosome 12 and confirmed a potential involvement of the *GXYLT1* and *SLCO1B1* genes in glycemic response to sulfonylureas. We report large clinical effects of variants in *SLCO1B1*, which encodes a transporter for sulfonylureas in the liver where it is metabolised, and report interaction with co-prescription of statins.

The SNP rs1234032 is an eQTL for *GXYLT1* in multiple tissues including whole blood. *GXYLT1* adds the first xylose to O-glucose-modified residues in NOTCH1 (31), which is a

major determinant of pancreatic islet cell mass and insulin secretion, and is a risk factor for diabetes (32). The C allele at rs1234032 was associated with increased expression of *GXYLT1*. Transgenic overexpression of human *GXYLT1* was previously shown to impair Notch signalling (39). Notch signalling pathway is known to play an important role in regulating development of pancreas and also shown to be expressed in adult pancreas (40). A recent study by Eom et al compared glucose levels, insulin secretion, islet and β -cell masses in Notch1 antisense transgenic (NAS) and control mice after intraperitoneal glucose tolerance test. This showed higher glucose levels, lower insulin secretion, decreased total islet and β -cell masses in NAS than the control mice. In line with this, we have shown increased trough glucose concentration with the C-allele in healthy volunteers who were naïve to type 2 diabetes medications who received a glipizide challenge and hence worse response.

The C allele at rs10770791 was significantly associated with reduced expression of *SLCO1B1* mRNA in the liver and worse glycemic response to sulfonylureas. *SLCO1B1* encodes the organic anion-transporting polypeptide, OATP1B1, which facilitates the hepatic uptake of clinically relevant drugs such as statins. Gliclazide, glipizide, glyburide (glibenclamide), glimepiride, tolazamide and tolbutamide were prescribed for the subjects in this study. Around 90% of the prescriptions in the GoDARTS were for gliclazide and glipizide was the main sulfonylurea in the PMET cohorts. While gliclazide and glimepiride are substrates of OATP1B1 (31, 34), glyburide is shown to be a substrate of both OATP1B1 and OATP1B3 (31, 34-36, 41, 42). However, there are conflicting reports about glipizide, which has been shown to be a substrate of OATP1B3, but not OATP1B1 (36). Here we showed that both glyburide and glipizide were substrates of OATP1B1 and OATP1B3. Further, we observed that OATP1B1-Ala174 (c.521C) had a significantly lower uptake of glyburide ($p < 0.002$) and a trend towards a lower uptake of glipizide ($p = 0.06$) compared to OATP1B1-Val174 (c.521T). Examination of other known missense variants (rs60140950 (p.Gly256Ala),

rs11045681 (p.Tyr311Ser), rs11045819 (p.Pro155Thr)) in the *SLCO1B3* and *SLCO1B3-SLCO1B7* regions that are in partial LD with rs10770791 showed no significant association. Taken together these results suggest that the pharmacogenetic mechanism for the effect of rs10770791 on sulfonylurea response is primarily a result of altered hepatic expression of *SLCO1B1* and to a lesser extent, *SLCO1B3*. Partial LD of rs10770791 with various missense variants may contribute to its effect on sulfonylurea response; however, conditional analysis demonstrated association of rs10770791 with glycaemic response independent of the missense variants. The reduced *SLCO1B1* expression likely results in less OATP1B1-mediated transport of sulfonylurea into the liver and potentially higher plasma concentrations available at the site of action (pancreas).

There is a high prevalence of multimorbidity and subsequent polypharmacy in type 2 diabetes, highlighting a need to consider drug-drug as well as drug-drug-gene interactions in prediction models of glycaemic response to sulfonylureas. Given that statins are often taken concomitantly with sulfonylureas with both being substrates of OATP1B1, we examined for a possible drug-drug-gene interaction and showed a significant interaction between statin use and *SLCO1B1* genotype (rs10770791) ($p=0.001$). Stratified analysis by statin use showed differential effects of rs10770791 in statin users and non-users. While the association between rs10770791 and glycaemic response to sulfonylureas was abolished in statin users, it was more pronounced in statin non-users. In those not treated with statins nearly a quarter of the population who carry two C alleles at rs10770791 had a 0.48% (5.2 mmol/mol) greater HbA1c reduction compared to T allele homozygotes. These large effects are the equivalent of starting a DPP4 inhibitor (43) and equated to a dose difference of 28 mg of gliclazide. Our findings suggest that the previous reported observational association between statins and hypoglycemia in sulfonylurea users (44) may be explained by interactions at *SLCO1B1*,

depending on the underlying genotype. The findings are consistent with previous studies in healthy volunteers and rodents demonstrating that atorvastatin administration is associated with increased levels of glimepiride (45) and glyburide (46), respectively. Given there is a strong recommendation to use statins by recent guidelines, statin use is increasing among people with diabetes (47). Therefore, integrating co-medications with genetic data could improve optimisation of polypharmacy regimens.

This study has some limitations. First, the modest sample size does not have sufficient power to detect the contribution of rare and low frequency variants in heritability estimation and/or glycemic response to sulfonylureas. However, this is the first GWAS and largest pharmacogenomic study on sulfonylureas response so far. Second, this study was conducted in whites of European descent, and therefore the results may not generalize to other populations. Third, even though we have performed several validation studies, direct replication of the findings in an independent study is warranted. Finally, further studies need to be done to elucidate the biological mechanism of the identified associations especially for *GXYLT1*.

In conclusion, we have established that common genetic variants contribute to the variation in glycemic response to sulfonylureas, with an estimated heritability of 37%. This result shows that a moderate proportion of the variance in glycemic response is genetic with an important role for common genetic variation in glycemic response to sulfonylureas. We report that a variant that modulates gene expression and circulating *GXYLT1* reduces response to sulfonylureas. We have also revealed a robust association between rs10770791, a *cis*-eQTL for *SLCO1B1* expression in the liver, and glycemic response to sulfonylureas, with reduced

SLCO1B1 expression associating with increased response to sulfonylureas. Our results suggest the potential of rs10770791 to be a biomarker for stratified medicine in diabetes. In addition, we have highlighted significant drug-drug-gene interactions between sulfonylurea, statin use and rs10770791, with clinically actionable genetic effects with pronounced differences in HbA1c reduction in a subgroup of patients treated with sulfonylureas without statins. Over the next 5 years we will see an ever-increasing availability of genotype or sequence data embedded in the medical records; given replication, the gene-statin interactions could be clinically actionable that will need to be taken into account at the point of prescribing sulfonylureas.

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A.E., Y.K., Y.M., M.K., C.N.A.P., J.C.F., M.M.H., L.M.H.; Manuscript writing: E.R.P., A.Y.D., S.W.Y., K.M.G. with contributions from all authors on the final version. A.Y.D., S.W.Y., E.R.P and K.M.G. are guarantors of this work and as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Data sharing: Summary-level data that underlie the results reported in this article will be deposited at the European Genome-phenome Archive and this will be available with publication.

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Tables and Figures

Tables

rsid	Chr	Position	Nearest gene	*EA	†NE A	‡EAF	§beta	se	p.value	N studies	Phet	N samples
rs1234032	12	42354629	<i>GXYLT1</i>	C	T	0.252	-0.141429	0.025	2.39×10^{-8}	7	0.55	4810
rs10770791	12	21338406	<i>SLCO1B1</i>	C	T	0.498	0.107475	0.020	4.80×10^{-8}	8	0.93	5476
rs2217693	12	21107376	<i>SLCO1B3- SLCO1B7</i>	G	A	0.925	-0.188639	0.037	8.40×10^{-8}	8	0.34	5479
rs8062936	16	52475969	<i>TOX3</i>	G	A	0.371	0.122292	0.023	1.57×10^{-7}	7	0.39	4810
rs7965567	12	21161025	<i>SLCO1B3- SLCO1B7</i>	T	G	0.051	0.251377	0.051	7.81×10^{-7}	6	0.57	4591
rs7703659	5	83222316	LOC107986386	A	G	0.132	-0.14596	0.030	1.15×10^{-6}	8	0.30	5478
rs1900362	13	85059600	<i>LINC00333</i>	G	A	0.339	-0.102358	0.021	1.26×10^{-6}	8	0.69	5475
rs11816402	10	61491043	MRLN	T	C	0.082	-0.217113	0.046	2.66×10^{-6}	7	0.39	4810
rs11667346	19	8817909	NFILZ	G	A	0.099	-0.194814	0.042	4.39×10^{-6}	6	0.37	4591
rs59012839	9	138419280	LCN1	G	A	0.097	-0.216643	0.047	4.51×10^{-6}	6	0.56	4210
rs12928694	16	10067543	<i>GRIN2A</i>	A	C	0.159	-0.123792	0.027	5.52×10^{-6}	8	0.56	5475
rs58013952	19	29917652	<i>LOC284395</i>	T	C	0.115	0.160896	0.036	5.78×10^{-6}	7	0.56	4810
rs75553467	1	74014130	LINC02238	C	G	0.059	-0.233071	0.052	6.31×10^{-6}	6	0.48	4591
rs73239453	4	14122932	<i>LINC01085</i>	T	C	0.106	0.160255	0.036	8.69×10^{-6}	7	0.93	4810
rs10250448	7	33489223	<i>BBS9</i>	G	A	0.10	0.15	0.03	8.94×10^{-6}	8	0.78	5479

*EA: Effective allele, †NEA: Non-effective allele, ‡EAF: Effective allele frequency, §A negative beta implies that the effective allele is associated with reduced response to sulfonylureas.

Table 1: Results for index variants in the top 15 independent loci ($p < 1.0 \times 10^{-5}$) associated with glycaemic response identified in a GWAS meta-analysis of sulfonylurea users with type 2 diabetes.

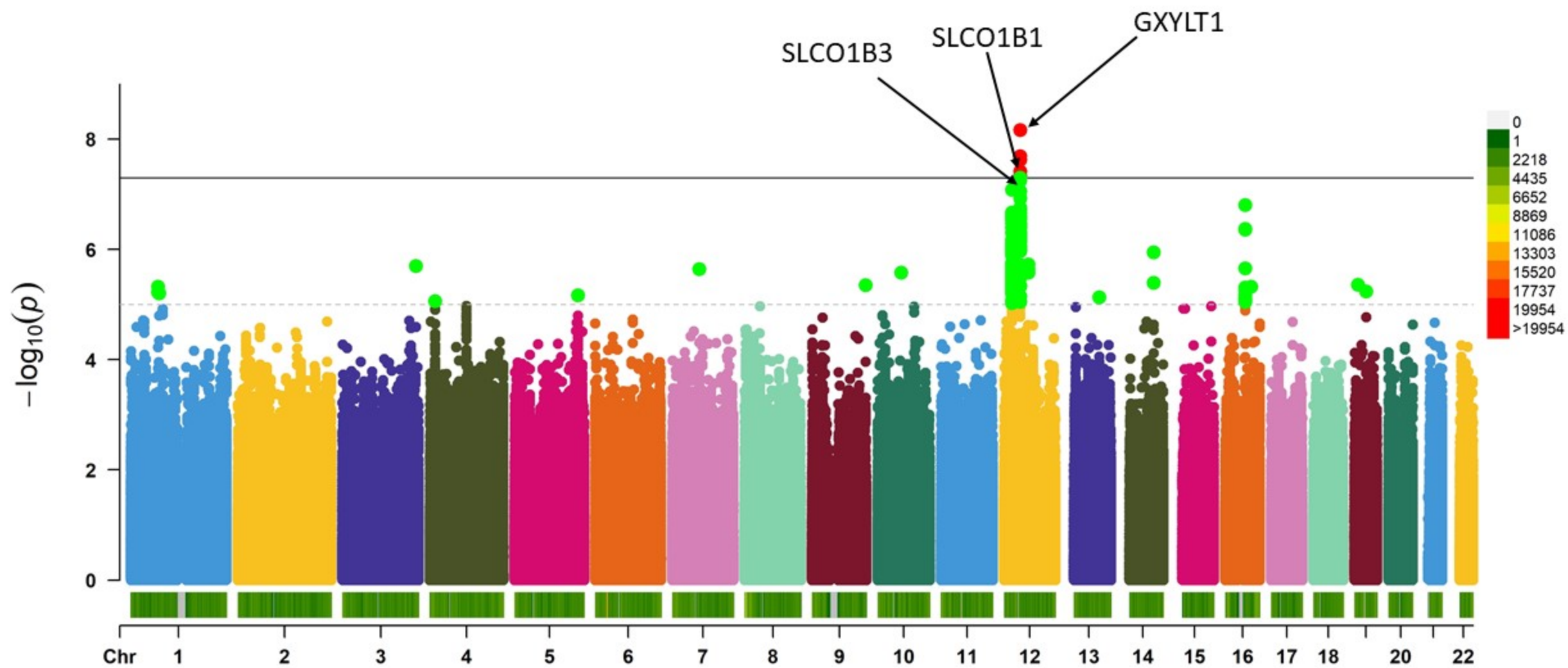
Figure legends

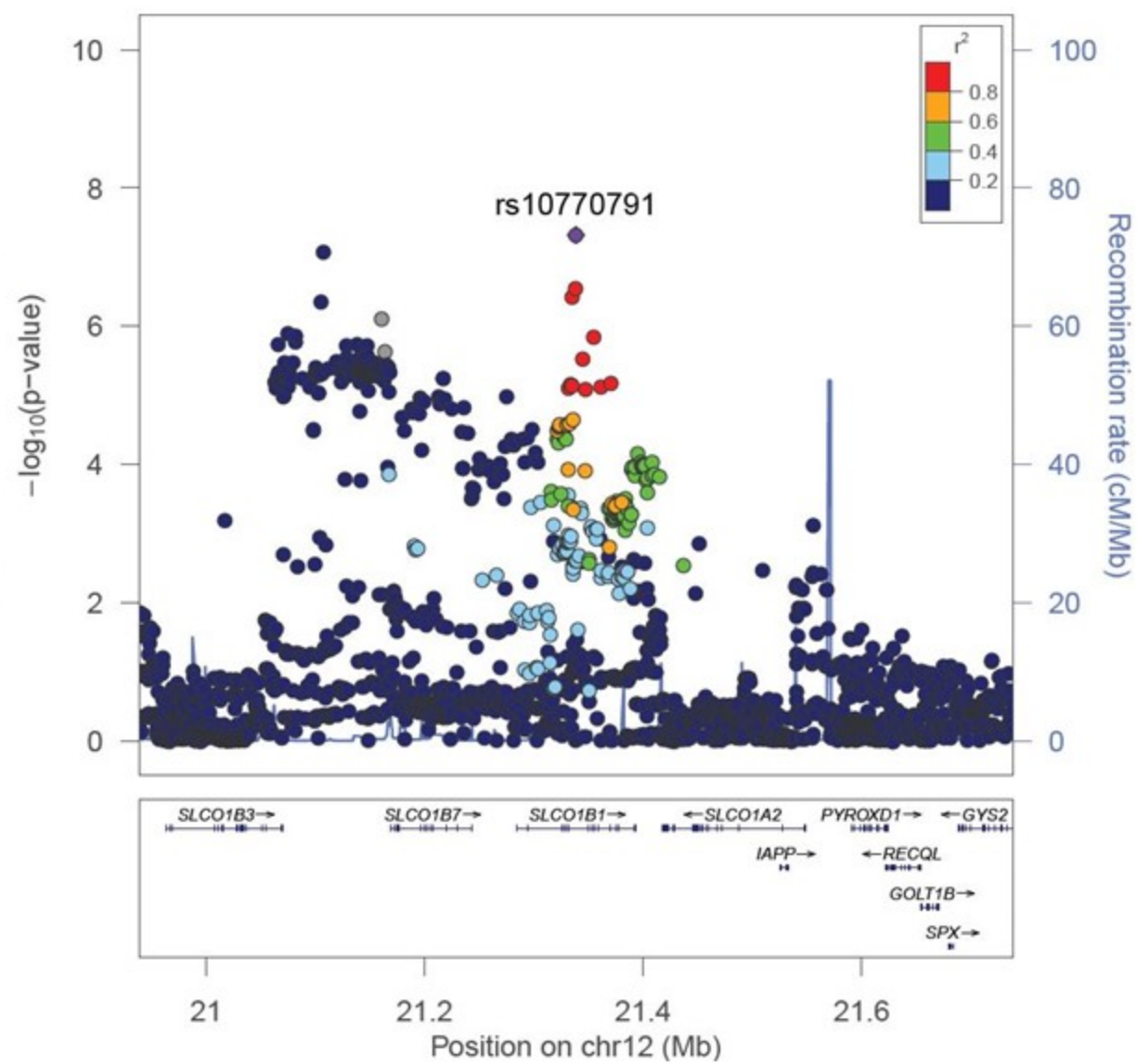
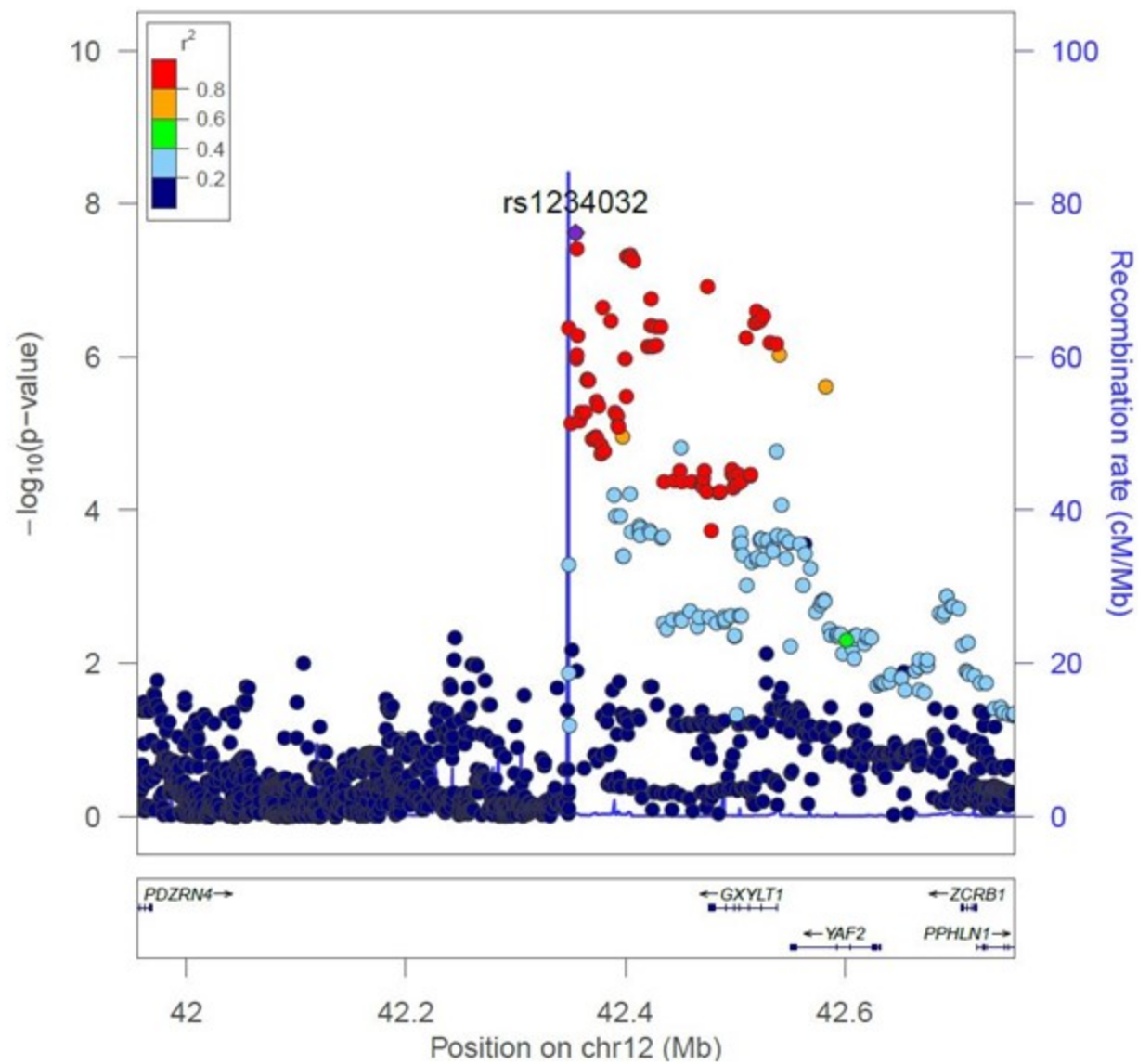
Figure 1: Manhattan plot of a genome-wide results from single marker association with glycemic response to sulfonylureas using an additive genetic model in a meta-analysis consisting of 5,485 individuals with type 2 diabetes on sulfonylureas.

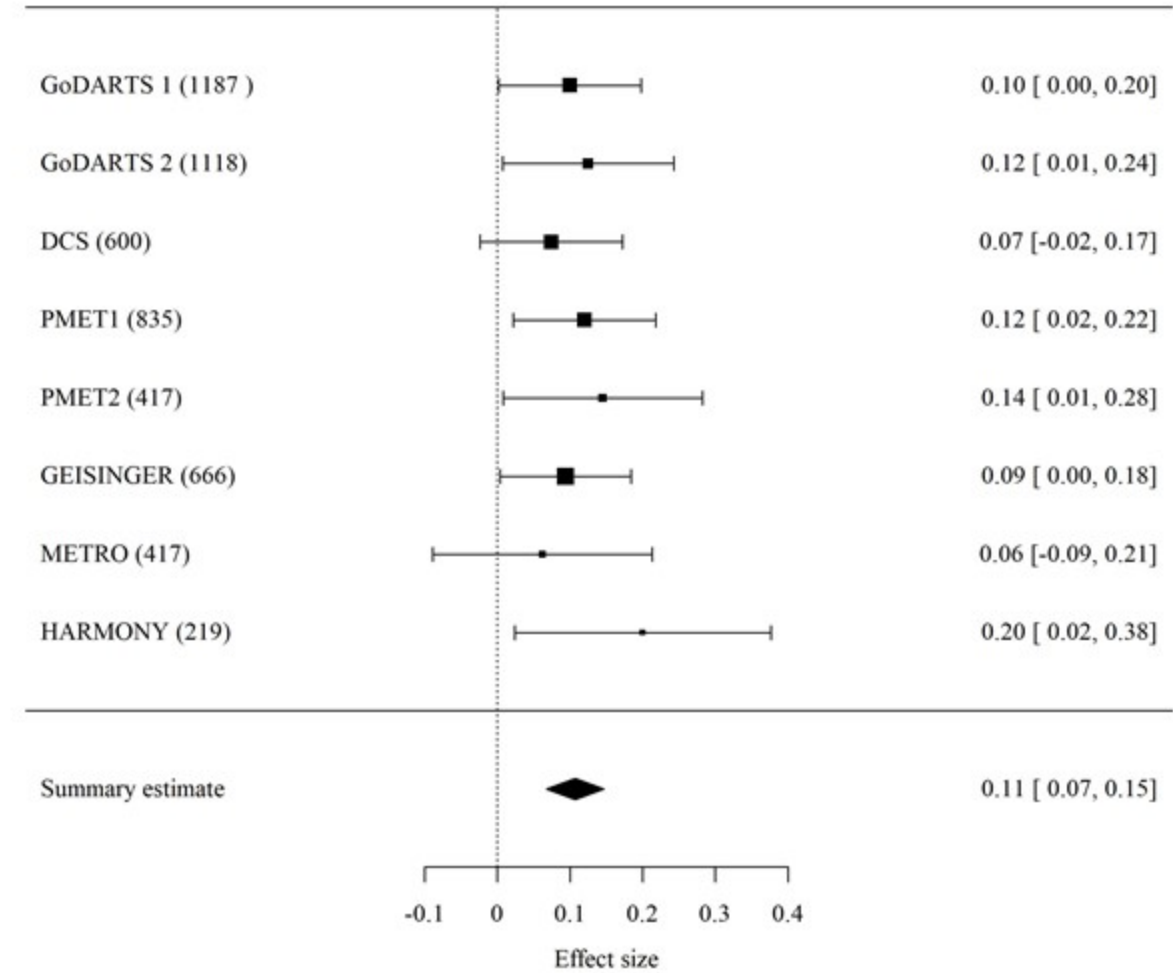
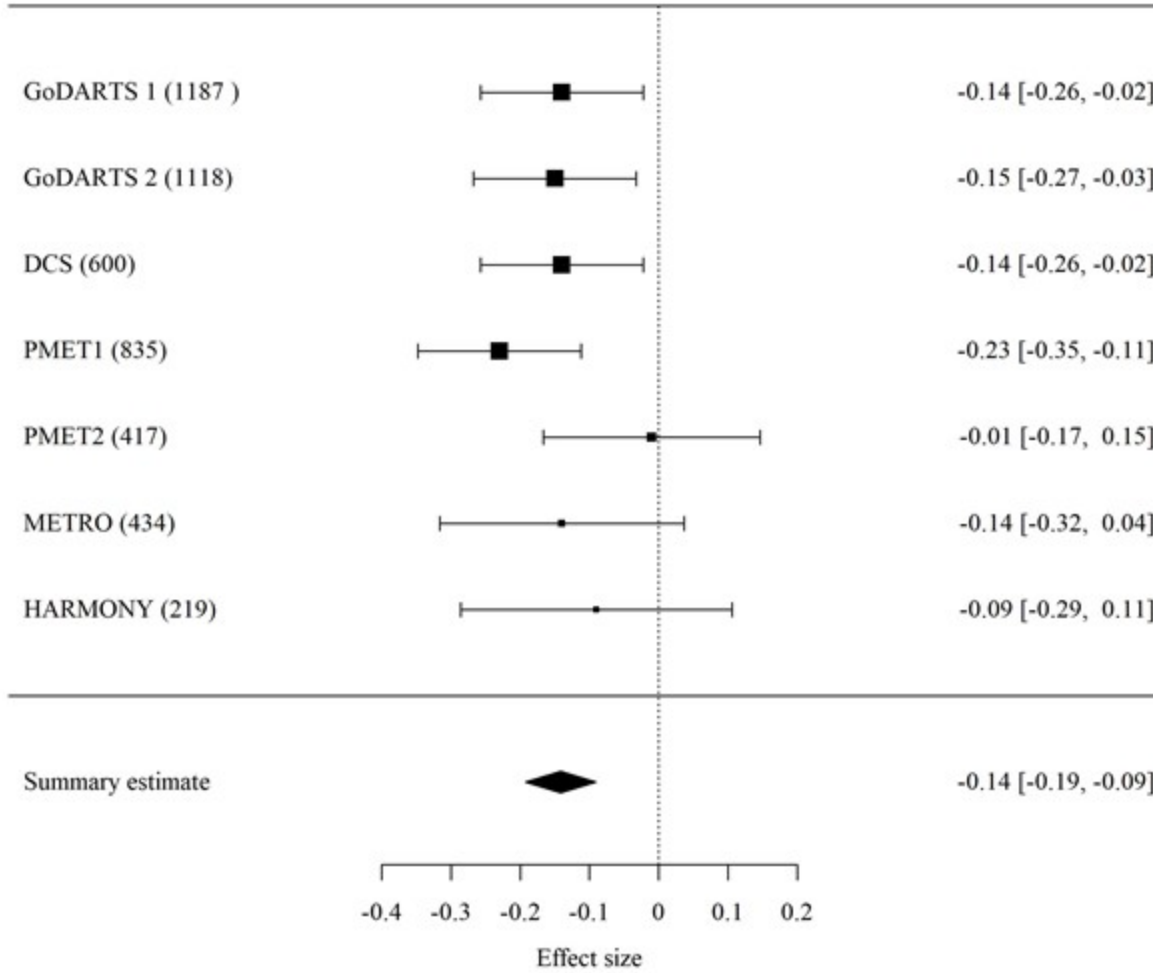
Figure 2: Regional association plots around genome-wide significant SNPs, rs1234032 (left) and rs10770791 (right) locus at chromosome 12 for the meta-GWAS. The purple diamonds in both plots indicate the top SNPs in the locus.

Figure 3: Forest plot of the meta-analysis of the association of HbA1c reduction with rs1234032 (left) and rs10770791 (right) variants after sulfonylurea treatment. Information on the various cohorts can be found in supplemental information. The numbers in parentheses indicate the number of individuals in each of the cohorts. The last column shows the effect size and [95% confidence interval]

Figure 4: Glyburide and glipizide uptake in HEK293-FlpIn cells recombinantly expressing SLCO1B1 or SLCO1B3. (A) Uptake of [3H]-glyburide and [3H]-glipizide in HEK293-FlpIn stable cells expressing vector only (EV), SLCO1B1 or SLCO1B3. Rifampicin (50 μ M) is used as a canonical inhibitor of SLCO1B1 and SLCO1B3 (Sudsakorn et al., 2020). P-values, representing significance from EV, were determined by one-way analysis of variance followed by Dunnett's two-tailed test. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Bars represent the mean \pm SEM uptake from three wells. Values shown are from a representative experiment of at least three independent studies. (B) Uptake of [3H]-estrone sulfate, [3H]- glyburide and [3H]-glipizide in HEK293-FlpIn stable cells expressing vector only, SLCO1B1 and SLCO1B1-V174A. Estrone sulfate is a canonical substrate of SLCO1B1 and is used as a positive control in this assay. P-values, assessing significance from EV, were determined by one-way analysis of variance followed by Dunnett's two-tailed test. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Bars represent the mean \pm SEM uptake from four wells from a representative experiment. The uptake values for [3H]- glyburide and [3H]-glipizide shown are from at least four independent studies with three or four replicates per study. (C) Inhibition of [3H]- glyburide uptake by atorvastatin and simvastatin acid in HEK293-FlpIn stable cells expressing SLCO1B1 and SLCO1B3. Each point represents the mean \pm SEM uptake from four wells. Values shown are from a representative experiment of two independent studies.

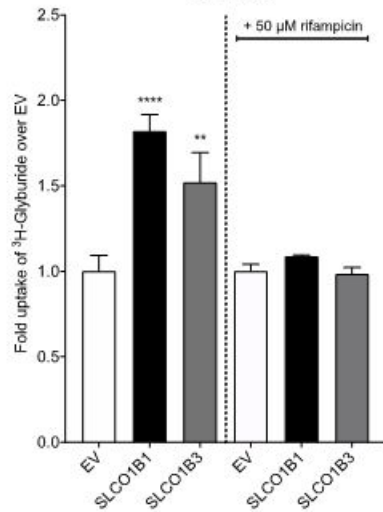




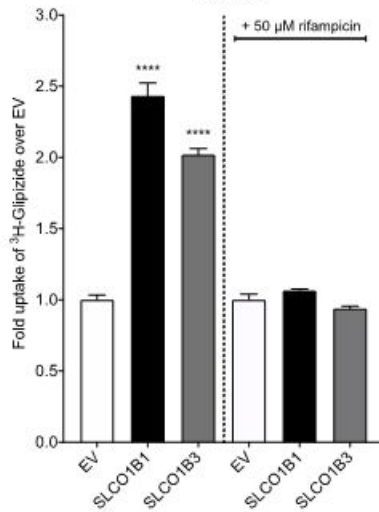


(A)

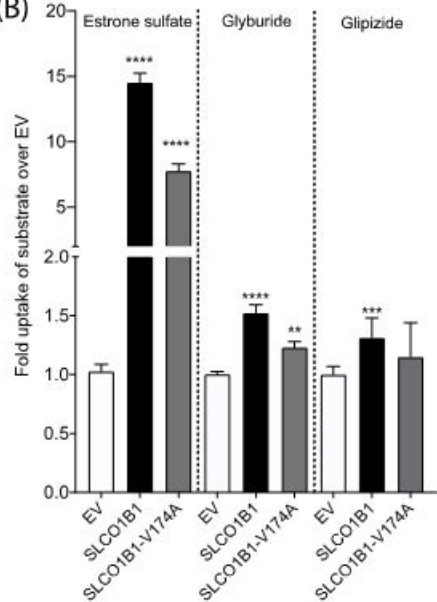
Glyburide



Glipizide



(B)



(C)

